

WHAT IS CLAIMED IS:

1. A method for estimating a melting temperature (T_m) for a polynucleotide at a desired ion concentration $[X^+]$, said polynucleotide having a known G-C content value, $f(\text{G-C})$, comprising:

- (a) obtaining a reference melting temperature (T_m^0) for the polynucleotide, said reference melting temperature being a melting temperature obtained or provided for the polynucleotide at a reference ion concentration $[X^+]_0$; and
- (b) modifying the reference melting temperature by a logarithm of the ratio of said desired ion concentration to said reference ion concentration, said logarithm being multiplied by a coefficient which is a function of the G-C content value,

wherein the estimated melting temperature is calculated using the reference melting temperature.

2. A method for estimating a melting temperature (T_m) for a polynucleotide at a desired ion concentration $[X^+]$, said polynucleotide having a known G-C content value, $f(\text{G-C})$, comprising:

- (a) obtaining a reference melting temperature (T_m^0) for the polynucleotide, said reference melting temperature being a melting temperature obtained or provided for the polynucleotide at a reference ion concentration $[X^+]_0$; and
- (b) modifying the reference melting temperature by an amount,

$$k(f(G-C)) \times \ln \frac{[X^+]}{[X^+]_0}$$

in which the coefficient $k(f(G-C))$ is a function of the G-C content value $f(G-C)$,
wherein the estimated melting temperature is obtained by using the reference melting
5 temperature.

3. The method of claim 2, wherein the coefficient k has a value determined by the
relation

$$k(f(G-C)) = m \cdot f(G-C) + k_0 ; \text{ and}$$

10 wherein a first coefficient, m and a second coefficient, k_0 , are optimized for predicting
polynucleotide melting temperatures T_m^0 .

4. The method of claim 2, wherein the reference melting temperature T_m^0 is used to
calculate T_m according to the formula:

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$$T_m = T_m^0 + k \times \ln \frac{[X^+]}{[X^+]_0} .$$

5. The method of claim 4, wherein the coefficient k

20 $k(f(G-C)) = m \cdot f(G-C) + k_0$; and wherein a first coefficient, m and a second
coefficient, k_0 , are optimized for predicting polynucleotide melting temperatures T_m^0 .

6. The method of claim 2, wherein the reference melting temperature T_m^0 is used to calculate T_m according to the formula:

$$T_m = T_m^0 + k(f(G - C)) \times \ln \frac{[X^+]}{[X^+]_0} + b \times (\ln^2[X^+] - \ln^2[X^+]_0).$$

5 7. The method of claim 6, wherein k is $m \cdot f(G - C) + k_0$; and wherein a first coefficient, m , a second coefficient, k_0 , and a third coefficient b are optimized for predicting polynucleotide melting temperatures T_m^0 .

10 8. The method according to claim 5, wherein m is -3.22, k_0 is 6.39.

9. The method according to claim 7, wherein m is -4.62, k_0 is 4.52 and $b = -0.985$.

15 10. The method of claim 2, wherein the reference melting temperature T_m^0 is used to calculate T_m according to the formula:

$$\frac{1}{T_m} = \frac{1}{T_m^0} + k(f(G - C)) \times \ln \frac{[X^+]}{[X^+]_0}.$$

20 11. The method of claim 10, wherein the coefficient k has a determined value by the relation $k f(G - C) = m \cdot f(G - C) + k_0$; and wherein a first coefficient, m and a second coefficient, k_0 are optimized for predicting polynucleotide melting temperatures.

20. The method of claim 1, wherein the polynucleotide ranges from about 10 to about 30 basepairs in length.

5 21. The method of claim 1, wherein the reference melting temperature is experimentally determined.

22. The method of claim 1, wherein the reference melting temperature is calculated from a theoretical model.

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23. The method of claim 1, wherein the reference melting temperature is obtained by utilizing a nearest neighbor model.

24. The method of claim 1, wherein the reference ion concentration is 1 M.

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25. The method of claim 1, wherein the ion is a monovalent ion.

26. The method of claim 1, wherein the ion is selected from the group consisting of the cations of sodium, lithium, potassium, rubidium, cesium and francium.

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27. The method of claim 1, wherein the desired ion concentration ranges between about 1mM and about 5M.

28. The method of claim 1, wherein the desired ion concentration ranges between about 10 mM and about 2M.

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29. The method of claim 1, wherein the desired ion concentration ranges between about 70 mM and about 1021mM.

30. A computer system for predicting a melting temperature, which computer
5 system comprises:
(a) a memory; and
(b) a processor interconnected with the memory and having one or more software
components loaded therein,
wherein the one or more software components cause the processor to execute steps of a method
10 according to claim 1.

31. A computer program product comprising a computer readable medium having
one or more software components encoded thereon in computer readable form, wherein the
one or more software components may be loaded into a memory of a computer system and
15 cause a processor interconnected with said memory to execute steps of a method according to
claim 1.